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# Limited Thrombogenicity of Low Temperature, Laser-Welded Vascular Anastomoses

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***Background and Objective:*** **Laser welding of vascular anastomoses has been shown to be feasible, but the clinically relevant advantages of this technique remain to be demonstrated.**
***Study Design/Materials and Methods:*** **To determine whether laser welding decreases initial anastomotic thrombogenicity, argon laser-welded aortic and venacaval anastomoses were constructed in 15 New Zealand white rabbits. Low temperature welding was done in one-half of welded anastomoses using low power (< 0.7W) and increased irrigation with saline during welding. Anastomotic surface temperature, bursting strength, procedure time, and surface thrombogenicity ($^{111}$Indium labeled platelet accumulation) were determined at 24 hours and compared to sutured anastomoses.**
***Results:*** **Aortic and vena caval low temperature laser-welded anastomoses were significantly less thrombogenic than sutured or higher temperature laser-welded anastomoses. In addition, bursting strength of welded anastomoses exceeded physiologic requirements and vessel closure time was less with welding than with suture techniques.**
***Conclusion:*** **Low temperature laser welding limits anastomotic thrombogenicity, which may improve early patency of venous and small arterial bypass grafts.** © 1996 Wiley-Liss, Inc.

**Key words: platelet adhesion, thermal injury, anastomotic welding**

## INTRODUCTION

Laser-assisted vascular anastomosis has been proposed as an alternative to standard suturing techniques in arterial reconstruction. Suggested benefits of laser anastomotic welding include less operating time, improved anastomotic compliance, unrestricted enlargement of growing vessels, and decreased foreign body reaction to suture material. However, initial strength of laser-welded anastomoses is less than sutured anastomoses [1], resulting in an increased risk of aneurysm formation. In addition, laser welding of vascular anastomoses is more complex and more expensive than standard suturing techniques [2]. Thus for laser anastomotic welding to supplant standard suturing techniques, improved acute and/or long-term patency of arterial reconstructive procedures must result from use of laser welding.

Acute failure of arterial bypass grafts is due to thrombus accumulation within the graft. Initially, the most thrombogenic portion of an arterial bypass done with autogenous vein conduit is the vein artery anastomosis [3]. Tissue trauma from suturing and use of thrombogenic suture material appear to be important factors contributing to the initial thrombogenicity of sutured anastomoses. Laser-assisted vascular anastomosis may reduce initial anastomotic thrombogenicity as tissue trauma from suturing is limited and laser thermal modification of vascular proteins appears to reduce platelet reactivity [4].

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This study examined the initial platelet reactivity of laser-welded and sutured anastomoses in both arteries and veins. In addition, the effect of limiting tissue temperature during laser welding on anastomotic thrombogenicity and bursting strength was investigated. Low temperature vascular anastomotic welding was shown to significantly limit initial anastomotic thrombogenicity without changing bursting strength, which could mean that laser welding of vascular anastomoses could improve initial patency of small arterial and venous bypass grafts.

## MATERIALS AND METHODS

Aortic and vena caval laser-welded and sutured anastomoses were done in 17 female New Zealand white rabbits (3–4.5 kg body weight). The protocol for this animal study was approved by the University of Florida Animal Research Committee and the Gainesville Veterans Administration Animal Research Committee. General anesthesia was induced using intramuscular Ketamine (35 mg/kg) and Xylazine (5 mg/kg). Anesthesia was maintained with inhalational Halothane following endotracheal intubation. Lactated Ringers solution was administered at 10 cc/kg per hour intravenously through an ear vein throughout the procedure, and normothermia was maintained with a heat lamp.

Following standard sterile surgical preparation of the abdominal skin, a midline laparotomy was performed to expose the aorta and inferior vena cava from the level of the renal vessels to the iliac bifurcation. K-type thermal couples (Omega Engineering, Stamford, CT) were then attached to each vessel adjacent to the location of the planned incisions with a 7-0 PTFE suture (W L Gore, Flagstaff, AZ) (Fig. 1). Animals were systemically anticoagulated with a single bolus of heparin (100 u/kg) and anastomoses were done sequentially in the aorta and vena cava, alternating randomly between the vessel in which the first anastomosis was done. A 1 cm longitudinal incision was made in the anterior wall of the vessel, adjacent to the previously placed thermal couple. Vessel closure was then done in one of three randomly selected ways: (1) standard running suture closure using 7-0 polypropolene suture, (2) laser anastomotic welding using standard techniques, and (3) laser anastomotic welding using techniques designed to limit anastomotic temperature during welding. Baseline surface temperature was recorded prior to beginning vessel closure and subsequently every 30 seconds thereafter until completion of the procedure.

In vessels closed using laser welding, traction sutures of 7-0 PTFE were placed beyond the corner of each incision to allow approximation of the vessel edges and 1–2 drops of fluorescein isothiocyanate dye solution (FITC) applied to the edges of the vessel incision (Fig. 1). FITC dye was made by mixing 10 mg of isomer I FITC, 10 ml of 0.9% sodium chloride irrigation solution (pH 4.5–7.0), and 1 ml of 8.4% sodium bicarbonate to make an orange solution. Argon ion laser energy was then delivered to the vessel edges through a 600 u quartz fiberoptic, which was maintained at 1 cm distance from the tissue and moved continuously back and forth across the anastomosis during welding. Laser energy was delivered continuously and power settings were 0.9 watts for the aorta and 0.7 watts for the inferior vena cava in animals in which standard welding techniques were used and 0.7 watts for the aorta and 0.5 watts for the inferior vena cava in animals in which lower temperature welding was attempted. Saline irrigation was used during all laser welding procedures, 1 cc/min in animals in which standard welding techniques were used and 3 cc/min in animals in which lower temperature welding was attempted. FITC dye was reapplied as necessary. Following completion of each anastomosis, the proximal clamp was released and any leakage was repaired by an additional stitch in the sutured anastomoses or by spot welding in the laser-welded anastomoses.

Blood (30 ml) was withdrawn from the inferior vena cava of 15 rabbits prior to constructing the anastomoses, and autologous platelets were labeled with $^{111}$Indium oxine as described by Heaton et al. [5]. Labeled platelets were returned to each animal following completion of anastomoses and adequate hemostasis. At 24 hours after completion of the initial procedure, the aorta and inferior vena cava were removed by transacting the vessel 5 mm proximal and 5 mm distal to the previous anastomosis, flushed with heparinized saline to remove any blood, and the radioactivity of each specimen measured in a gamma well counter. Platelet accumulation on the anastomoses was expressed as $^{111}$Indium counts per minute per square millimeter of tissue.

After counting, bursting strength of the anastomoses was measured in the vessels from these 15 animals by cannulating the vessel with pressure tubing to which a pressure gauge was connected through a T connector. The opposite

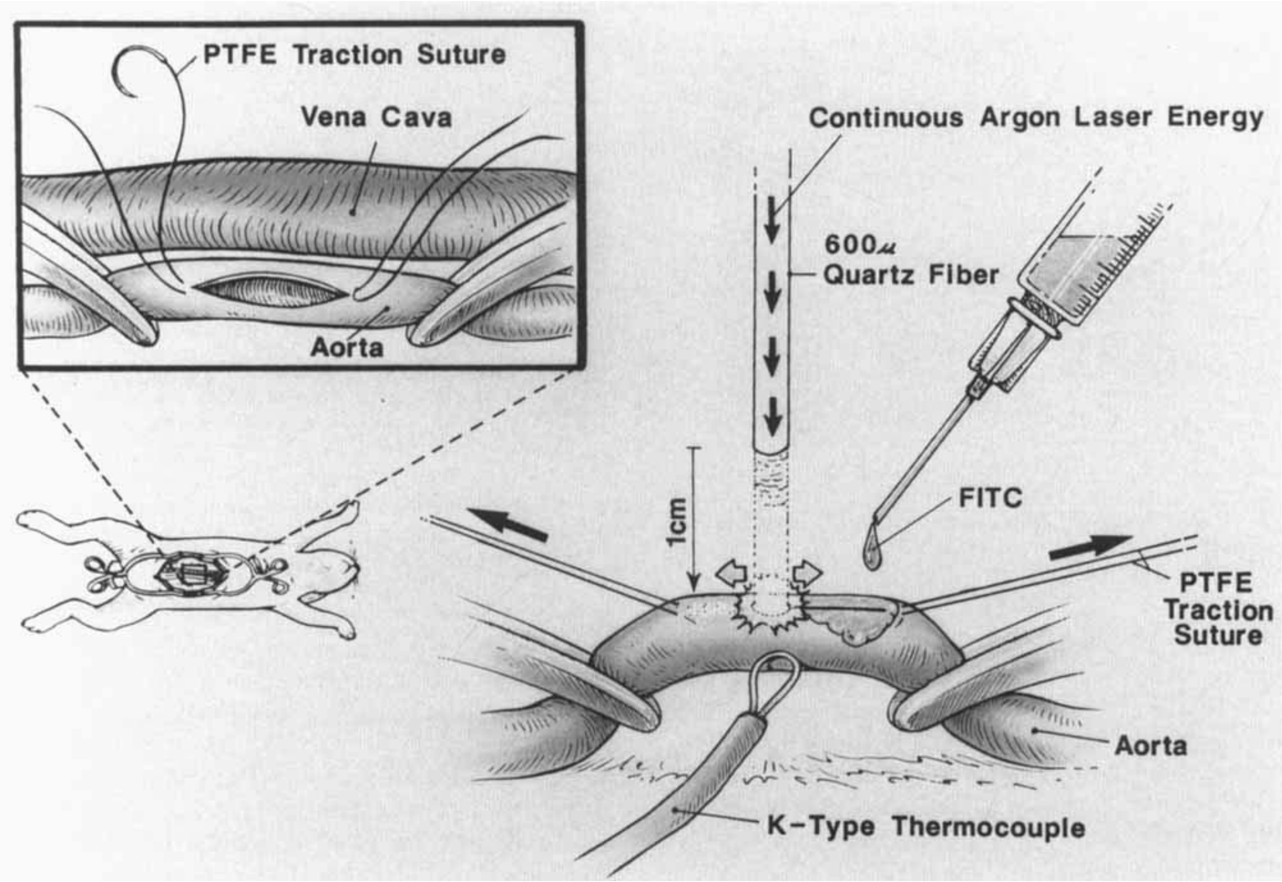


Fig. 1. Schematic of laser-welding procedure. Vessel closure was done using continuous wave argon laser energy after application of fluorescein isothiocyanate dye. Anastomotic surface temperature during welding was measured using a K-type thermocouple.

end of the vessel was then occluded and intraluminal pressure gradually increased by injecting saline into the vessel. Anastomotic burst failure point was recorded as the pressure at which the anastomosis ruptured.

In the remaining two animals, two laser-welded anastomoses were done in the aorta and two in the vena cava, one using standard techniques and one using the techniques designed to reduce tissue temperature during welding. Platelet labeling was not done in these animals. Rather, at 24 hours after welding, the aorta and vena cava were prepared for histology by in situ perfusion fixation at 100 mm Hg using 1% gluteralderhyde. Fixed specimens were then sectioned, stained with hematoxylin and eosin, and examined under light microscopy.

Differences in anastomotic platelet reactivity, bursting strength, temperature during anastomotic closure, and time required to complete the anastomosis between the three different types of vessel closures were analyzed by comparison of group means using analysis of variance and the unpaired Student's t-test. Data are expressed as mean ± one standard deviation and mean values were considered different if P values were $<0.05$.

## RESULTS

### Temperature

Average anastomotic surface temperature during laser welding of aortic and inferior vena caval closures was 36.6 ± 2.2°C and 34.5 ± 1.1°C, respectively, in vessels in which lower laser power and increased saline irrigation of the anastomotic area were used during welding. Maximum temperature during welding using these techniques was 44.0 ± 6.3°C for the aorta and 39.5 ± 3.2°C for the vena cava. These temperatures were significantly lower ($P < .05$) than similar surface temperatures from aortic and vena caval laser welding procedures done without

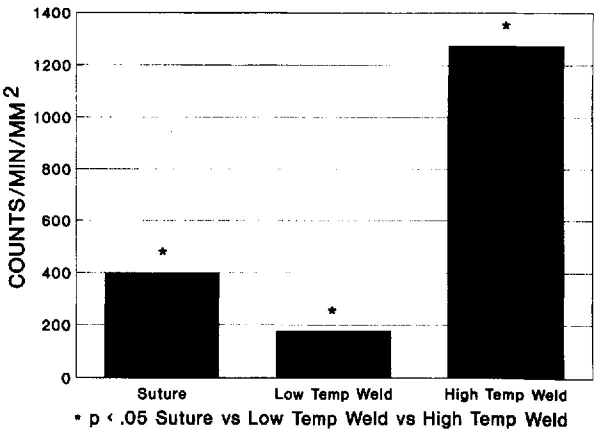


Fig. 2. Labeled platelet accumulation on aortic anastomoses 24 hours after vessel closure. Anastomoses done using techniques that limited surface temperature during laser welding were significantly less thrombogenic.

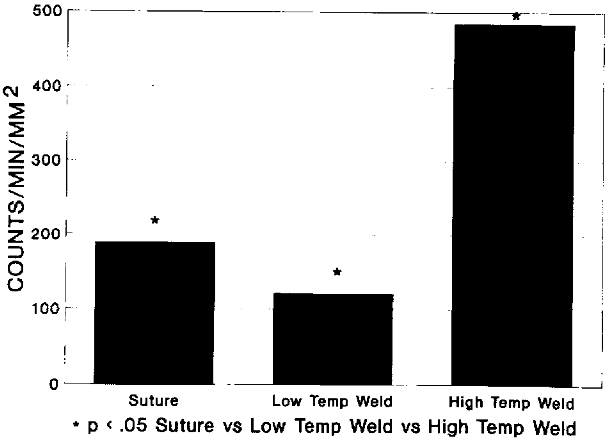


Fig. 3. Labeled platelet accumulation on vena caval anastomoses 24 hours after vessel closure. Anastomoses done using techniques that limited surface temperature during laser welding were significantly less thrombogenic.

lower laser power and increased saline irrigation (average aortic temperature 42.9 ± 3.3°C, maximum temperature 52.5 ± 7.2°C, average vena caval temperature 43.5 ± 2.4°C, maximum temperature 53.4 ± 4.0°C).

## Thrombogenicity

Anastomotic thrombogenicity as assessed by platelet accumulation at 24 hours was significantly influenced by the surface temperature of the anastomosis during welding and by the type of vessel closure used. (Figs. 2 and 3) Laser-welded anastomoses done using efforts to minimize tissue temperature during welding (low temperature anastomoses) were significantly less thrombogenic than sutured aortic and venous anastomoses. In contrast, laser-welded anastomoses done without such efforts (high temperature anastomoses) were significantly more thrombogenic.

## Anastomotic Bursting Strength

The pressure at which sutured aortic anastomoses failed exceeded 300 mm Hg in all cases. The pressures at which both low temperature and high temperature laser-welded aortic anastomoses failed were lower (Fig. 4) but mean bursting pressure for both types of aortic laser-welded anastomoses were not statistically different from the mean value for sutured anastomoses. In addition, despite the lower bursting strength of laser-welded aortic anastomoses, no evidence of anastomotic bleeding or aneurysm formation was seen. In contrast, mean bursting pressure of both low temperature and high temperature laser-welded vena caval anastomoses was significantly lower than the bursting pressure of laser-welded vena caval anastomoses (Fig. 4). Again, despite the significantly lower bursting pressure of laser-welded vena caval anastomoses, no evidence of perianastomotic bleeding or aneurysm formation was seen.

## Procedure Time

Time required for vessel closure was significantly less in laser-welded anastomoses compared to sutured anastomoses (Fig. 5). However, the total time the vessel was occluded during incision, and closure was significantly longer when laser welding was done. This was due to the increased time required for vessel preparation and laser setup during laser welding.

## Histology

Histologic examination revealed more extensive thermal injury at higher temperature laser-welded anastomoses compared to anastomoses done using techniques that lowered tissue temperature during welding (Fig. 6). A coagulum of red blood cells and fibrin was seen on the edges of higher temperature anastomoses and significant amounts of mural thrombus were present on the luminal surface of the anastomoses. Vacuolation of the adventitum was also seen, and leukocytes, some of which had margination, were common in areas of extensive injury. In contrast, in lower temperature anastomoses, less fibrin coagulum and cellular debris were present and fewer white

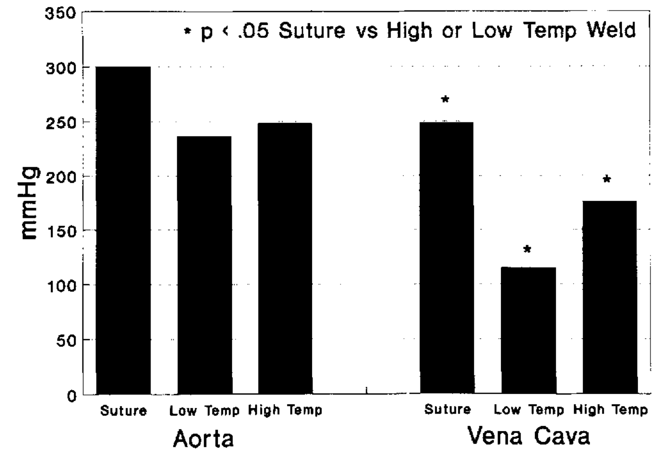


Fig. 4. Bursting strength of aortic and vena caval anastomoses. Aortic anastomoses had equivalent strength, regardless of the type of closure, whereas laser-welded vena caval anastomoses were weaker than sutured anastomoses.

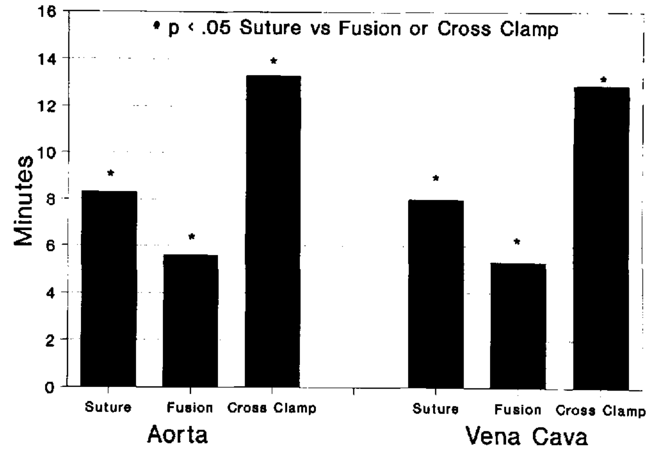


Fig. 5. Time required to complete aortic and venal caval anastomoses. *Suture*—total time required for sutured anastomoses, *Fusion*—time required for vessel closure using laser welding, *Cross clamp*—total time required for laser-welded anastomoses, including vessel preparation, laser welding, and repeat laser application to close any bleeding sites. Time required for vessel closure using laser welding was significantly less than time required for suture closure but total time required for laser welding was significantly longer.

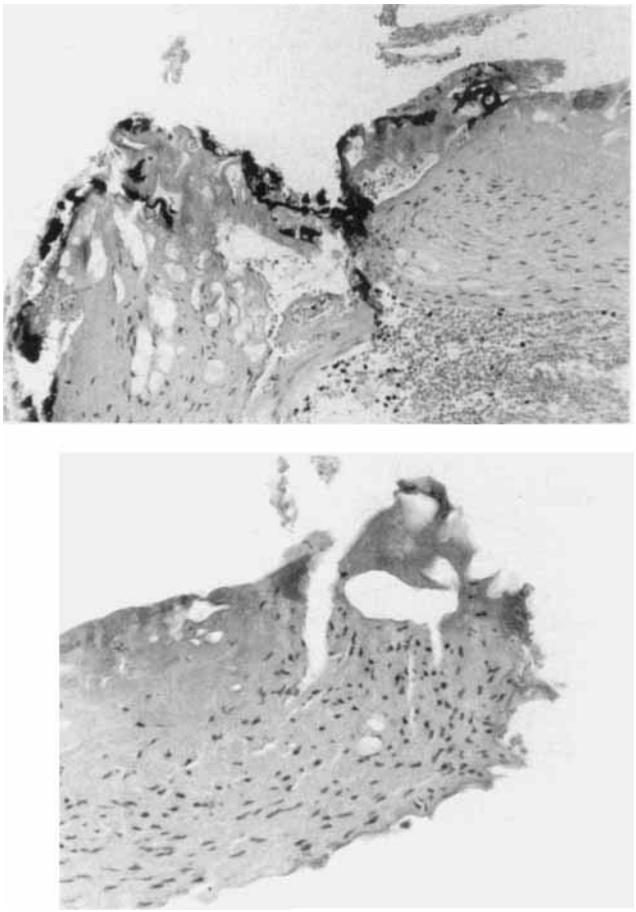

Fig. 6. Photomicrograph of high temperature (top) and low temperature (bottom) aortic laser-welded anastomoses (hematoxylin and eosin, magnification 312x.) A coagulum of red blood cells and fibrin is seen on the edges of the higher temperature anastomoses and significant amounts of microthrombus are present on the luminal surface. A significant number of leukocytes are also seen in the adjacent tissue. In contrast, less fibrin coagulum, cellular debris and fewer white cells are seen in the lower temperature anastomoses.

blood cells were seen. These changes were most prominent in laser-welded aortic anastomoses, whereas signs of thermal injury were less common and less intraluminal thrombus was seen in laser-welded venous anastomoses.

## DISCUSSION

Previous work by Fujitani et al. [6] has also demonstrated a decrease in labeled platelet accumulation on laser-welded arterial anastomoses compared to sutured anastomoses. In addition, they observed lower levels of platelet accumulation on laser-welded venous anastomoses compared to sutured venous anastomoses, although that difference did not achieve statistical significance. Laser welding was done in Fujitani's study [6] with levels of laser energy and irrigation comparable to those used for low temperature anastomoses in the present study. In addition, maximum anastomotic surface temperature reported by Kopchok et al. [7] using the welding techniques described by Fujitani et al. [6] was 44.2 ± 1.6°C, equivalent to the 44.0 ± 6.2°C maximum anastomotic temperature observed in the low temperature aortic anastomoses reported here. In

contrast, laser-welded vascular anastomoses done using techniques that resulted in higher tissue temperatures were found to be significantly more thrombogenic than those done with standard suturing techniques. Similarly, Cikrit et al. [8] have reported that laser-welded arterial anastomoses done using high levels of $CO_2$ laser energy are associated with a high incidence of acute vessel occlusion, suggesting increased anastomotic thrombogenicity. Kopchok et al. [7] have reported that laser welding done with $CO_2$ laser energy produces anastomotic temperatures of over 80°C and full thickness coagulation necrosis of the anastomotic tissue can occur during $CO_2$ laser welding [9]. Significant thermal tissue injury also occurred when higher temperature laser-welded anastomoses were done using argon laser energy in the present study. Thus it appears that anastomotic temperature during laser welding and anastomotic thermal tissue injury are important determinants of laser-welded anastomotic platelet reactivity, although the precise mechanism by which this occurs is unclear.

Initial strength of both low and high temperature laser-welded arterial and venous anastomoses was less than sutured arterial or venous anastomoses, similar to the results of previous reports by White et al. [10,11]. However, differences in anastomotic tissue temperature during welding within the temperature range reported here did not influence initial anastomotic strength. Argon laser welding has been reported to be secondary to collagen cross-linking without protein denaturation [12], and this process has been shown to occur at the low anastomotic surface temperatures observed in the present study when low temperature anastomoses were done. Use of FITC in the current study also may have facilitated low temperature tissue welding as Chuck et al. [13] have reported vessel welding to require three times less thermal laser energy when FITC is used. Progression to charring, which limits welding, also is slower with use of this dye.

Use of fibrinogen soder [14] or fibrin glue [15] has been reported to significantly increase the strength of laser arterial welds so that initial bursting strength is equivalent to the initial strength of sutured anastomoses. Whether use of these thrombogenic proteins will affect anastomotic platelet reactivity is unknown. However, Oz et al. [14] have reported that fibrinogen soder is platelet reactive immediately after welding, and Grubbs et al. [15] have demonstrated that leakage of the fibrin glue through the anastomosis during welding can cause vessel thrombosis. Therefore, the overall value of fibrinogen soder or fibrin glue in laser welding remains unclear. In addition, the degree of tissue bonding that occurs at low anastomotic temperature without use of soder appears to be sufficient to resist the physiologic pressure as previous studies by White et al. [10,11] and the results of the present study demonstrate no evidence of anastomotic bleeding or aneurysm formation in low temperature laser-welded vascular anastomoses.

Lawrence et al. [2] have pointed out that laser welding of arterial anastomoses is more time-consuming and more complex than standard suture techniques. Similar results were observed in the present study as overall vessel cross-clamp time was longer in vessels closed using laser welding. These observations in addition to the concerns about the initial strength of laser-welded anastomoses re-emphasize that improved initial and long-term patency and/or function of vascular reconstructions must be documented to justify clinical use of laser welding. The present study has shown that laser welding done using techniques that limit anastomotic temperature reduces anastomotic thrombogenicity, which could improve initial patency of vascular reconstructions. In addition, Quigley et al. [16] have shown that laser welding is associated with decreased anastomotic intimal hyperplasia compared to sutured anastomoses at 2 weeks, and Kuroyanagi et al. [17] reported essentially no anastomotic intimal hyperplasia in laser-welded arterial anastomoses examined at 1 year. Only preliminary results from studies of arterial laser welding in humans have been reported by White et al. [18] so that whether these observations from animal studies will be confirmed in humans remains to be seen. However, the limitation of initial anastomotic thrombogenicity using low temperature laser welding and the potential of limiting or eliminating anastomotic intimal hyperplasia support continuing study of this technique as a method of improving results of arterial reconstructive surgery, particularly in the difficult areas of venous reconstruction or bypass grafting of very small arteries.

## ACKNOWLEDGMENTS

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